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Acute infection with *Strongyloides venezuelensis* increases intestine production IL-10, reduces Th1/Th2/Th17 induction in colon and attenuates Dextran Sulfate Sodium-induced colitis in BALB/c mice

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ABSTRACT

Helminth infection can reduce the severity of inflammatory bowel disease. However, the modulatory mechanisms elicited by helminth infection are not yet fully understood and vary depending on the experimental model. Herein we evaluated the effect of acute infection of BALB/c mice with Strongyloides venezuelensis on the clinical course of ulcerative colitis induced by Dextran Sulfate Sodium (DSS) treatment of these animals. For the experiments, *S. venezuelensis*-infected BALB/c mice were treated orally with 4% DSS solution for seven days. As controls, we used untreated *S. venezuelensis* infected, DSS-treated uninfected, and untreated/uninfected BALB/c mice. During DSS treatment, mice from the different groups were compared with regards to the clinical signs related to the severity of colitis and intestinal inflammation. Mice acutely infected with S. venezulensis and treated with DSS had reduced clinical score, shortening of the colon, and tissue inflammation. Moreover, DSS-treated and infected mice showed reduced IL-4, INF-γ, and IL-17 levels and increase of IL-10 production in the colon and/or in the supernatant of mesenteric lymph nodes cell cultures that resulted in lower eosinophil peroxidase and myeloperoxidase activity in colon homogenates, when compared with DSS-treated uninfected mice. DSS-treated infected mice also preserved the intestine architecture and had normal differentiation of goblet cells and mucus production in the colon mucosa. In conclusion, the data indicate that the clinical improvement reported in DSS-treated infected mice was accompanied by the lower production of Th1/Th2/Th17 pro-inflammatory cytokines, stimulation of IL-10, and induction of mucosal repair mechanisms.

1. Introduction

Inflammatory bowel diseases (IBDs), such as Crohn's disease and ulcerative colitis, are chronic inflammatory diseases of the gastrointestinal tract that affect four to five million people around the world [1,2]. Although the multi-factorial etiology of these diseases is not yet fully understood, some studies have indicated that IBDs result from the induction of an inappropriate and/or uncontrolled immune response to intestinal antigens in genetically susceptible individuals, which leads to chronic intestinal inflammation [3,4]. IBD patients often experience relapsing episodes of intestinal inflammation resulting in symptoms such as abdominal pain, continuous or intermittent diarrhea, rectal bleeding, fever, fatigue, and weight loss, all of which hugely impacts patient's quality of life [5–7].

Abbreviations: DSS, Dextran Sulfate Sodium; IBD, Inflammatory bowel diseases; Th, T helper; DNBS, dinitrobenzene Sulfonic Acid; TNBS, Trinitrobenzene Sulfonic Acid; RTC, Randomly Clinical Trials; SPF, Specific Pathogen-Free; PBS, Phosphate-Buffered Saline; i.p., Intraperitoneal; MLN, Mesenteric Lymph Nodes; EPO, Eosinophil Peroxidase; MPO, Mieloperoxidase; HE, Hematoxylin-Eosin; PAS, Periodic Acid-Schiff; ConA, Concanavalin-A; IL, Interleukin; IFN-γ, Gamma Interferon; TGF-β, Transforming Growth Factor Beta; ELISA, enzyme-linked immunosorbent assay; OD, Optical Density; DPI, Days Post-Infection; UC, Ulcerative Colitis; NKT, Natural Killer T; Ig, Immunoglobulin

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The conventional therapies for IBD patients rely on long term prescription of aminosalicylates, corticosteroids, immunomodulators and/or antibiotics have no curative effects in most cases and can lead to serious side effects [8–11]. Therefore, it is necessary to understand the mechanisms involved in the induction and control of the intestinal inflammation observed in IBD patients to contribute for the development of better therapeutic strategies.

The drastic increase in the prevalence of chronic inflammatory diseases observed in developed countries in the late 20th century cannot be justified by genetic susceptibility, thus suggesting a strong role of environmental factors such as air and water pollution, diet, smoking, stress, and infectious diseases [1,12,13]. Chronic helminthic infections, whose incidence have significantly reduced in developed countries due to sanitation and medical improvements, are well-known inducers of immunomodulatory mechanisms [14–16]. Moreover, epidemiological data indicate that chronic helminth infections are inversely associated with the prevalence and the severity of IBDs [12,17,18].

Experimental data indicate that helminth infection or inoculation of worm-derived antigens of *Hymenolepis diminuta* [19], *Trichinella spiralis* [20,21], *Heligmosomoides polygyrus* [22], *Schistosoma mansoni* [23], *Ancylostoma caninum* [24], *Trichuris muris* [25], *A. ceylanicum* [26], *Anisakis simplex* [27], *Trichinella papuae* [28], *Echinococcus granulosus* [29], and *Ascaris lumbricoides* [16] ameliorate the severity of the clinical signals of the chemically induced colitis in mouse models. Helminth-induced modulatory mechanisms include the activation of Th2 cells and consequent inhibition of Th1 responses related to the severity of some forms of IBD [20–22,30] as well as induction/recruitment of Treg-cells to the gut, thus helping to maintain the intestine's tolerance state [16,30]. Recent data suggest that helminth infection alters the gut microbiome in mice and rats. For instance, *H. polygyrus* and *T. muris* infection increase the presence of members of the bacterial family *Lactobacillaceae* and reduce the abundance of *Eubacterium/Clostridium species* [31–33], *Lactobacillus* sp. has a protective effect in Dextran Sulfate Sodium (DSS) or trinitrobenzene sulfonic acid (TNBS)-induced ulcerative colitis [34,35].

In spite of the above mentioned evidence, the helminth modulatory effect in IBD is not always observed [36]. Indeed, Wang et al. [37] described that experimental infection with *H. diminuta* aggravated oxazolone-induced colitis in mice. In addition, another study reported that *T. muris* infection accelerated the progression of colitis in *Mdr1a-/-*, a mouse model of genetic susceptibility to IBD [38].

Clinical studies involving a small number of IBD patients treated with repeated ingestion of *Trichuris suis* eggs, a pig whipworm that causes a transient infection in man, reported that the infection alleviates the severity of the colitis in a group of the patients [39–41]. However, a systematic review of Randomly Clinical Trials (RTC) using *T. suis* eggs to treat IBD patients indicated that the evidence so far does not allow any definite conclusions regarding the efficacy and safety of using helminths to treat patients with IBD [42].

Given that the effect of helminth infection on IBD modulation is still controversial, and that immune mechanisms vary depending on the helminth species, the developmental phase, and the parasite burden [43] we sought to identify the modulatory effect of *Strongyloides venezuelensis* acute infection on DSS-induced colitis in mice. *S. venezuelensis* naturally infects rodents and the adult worms establish in the small intestine mucosa. The infection induces predominantly type-2 immune response in mice, which is responsible for complete elimination of the worm after 14 days [44,45]. Moreover, IL-10 production induced by the parasite has been associated with the modulation of ovalbumin-induced airway hyperresponsiveness in mice [46]. and type I diabetes induced by Streptozotocin [47]. In addition, *S. stercoralis* infection inversely correlates with type II diabetes in a human population [48]. The intestinal habitat of the parasite outside the colon, the short duration of the biological cycle of this nematode, its easy maintenance and laboratory manipulation, and the type of immune response it induces make this parasite an interesting subject to study the modulatory effect promoted by helminth infections in the evolution of ulcerative colitis in a murine model.

Our data shows that *S. venezuelensis* acute infection modulates DSS-induced ulcerative colitis in mice. The clinical improvements found in DSS-treated infected mice were accompanied by a significant reduction of colon inflammation and stimulation of mucosa repair mechanisms.

2. Material and methods

2.1. Mice

Specific pathogen-free (SPF) 8 to 9 weeks-old female BALB/c mice were provided by the Animal Facility of the Biological Sciences Institute of the Universidade Federal de Minas Gerais (UFMG, Brazil). During the experimental procedure, animals were housed in ventilated racks at the Animal Facility for Helminth Infected Animals of the Parasitology Department (UFMG, Brazil), fed with laboratory chow (Presence, Paulínia, SP, Brazil), and provided with tap water *ad libitum*. Experimental procedures used in this work received prior approval from the local animal ethics committee (CEUA - UFMG protocol number 129/2011).

2.2. Strongyloides venezuelensis infection

We used the nematode *S. venezuelensis*, originally isolated from wild *Rattus norvegicus* [49] that had been maintained in laboratory conditions by serial passage in Wistar rats. *S. venezuelensis* infective filiform larvae (L3) were obtained from vermiculite cultures of infected-rat feces and isolated using a Baermann apparatus described by Negrão-Corrêa et al. [45]. The recovered larvae were filtered, washed several times in phosphate-buffered saline (PBS - 13.7 mM NaCl, 0.27 mM KCl, 0.14 mM KH2SO4, and 0.43 mM Na2HPO4·7H2O), and counted. Each mouse was subcutaneously inoculated in the abdominal region with 200 µl of PBS containing 700 infective larvae (experimental day zero).

2.3. Induction of colitis and experimental design

Experimental colitis was induced by oral ingestion of DSS solution (DSS, 35–45 kDa, TdBConsultancy, Sweden) as described by Cooper et al. [50]. Briefly, DSS was added to filtered tap water at a concentration of 4% and offered to the mice *ad libitium* for seven consecutive days, starting at experimental day 5. Fresh DSS solution was prepared and replaced daily.

For the experiments, BALB/c mice were randomly divided into four groups: infected (1) and non-infected (2) control groups, which received only tap water, and infected (3) and non-infected (4) DSS-treated groups, which received a 4% DSS aqueous solution as the only source of liquid from day 5 to 12 of the experimental procedure (Fig. 1). Animals were weighed daily and examined with regards to clinical appearance, fecal consistence, and rectal bleeding. At days 9 and 12, five-seven animals from each experimental group were anaesthetized with intraperitoneal (i.p.) injection of ketamine/xylazine [88 mg Dopalen/kg (Sespo Industria e Comercio Ltda, Brazil) and 16 mg Kensol/kg (Laboratórios Konig S.A., Argentina)], and euthanized by cervical dislocation. Mesenteric lymph nodes (MLN) were collected under sterile conditions and the cells cultivated *in vitro* for cytokine production measurement. The small intestine of each infected mouse was collected for evaluation. The colon was carefully separated, measured, and longitudinally opened to observe possible microscopic lesions [51,52]. The distal half of the colon (portion that is mostly affected by DSS-treatment [53,54] was kept at −20 °C for measurement of cytokines and enzymatic activities (eosinophil peroxidase and mieloperoxidase). Five additional mice from each group were anaesthetized and euthanized at the end of the experimental procedure (day 12), and their colons isolated and processed for histopathological analysis.